Water, a unique medium for organic reactions
Buurma, Niklaas

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Hydrolysis of Activated Amides Around the Temperature of Maximum Density of Water – Isochoric Conditions

At temperatures above and below the temperature of maximum density, TMD, for water at ambient pressure, pairs of temperatures exist at which the molar volumes of water are equal. First-order rate constants for the pH-independent, water-catalysed hydrolysis of 1-benzoyl-1,2,4-triazole (6.1) in very dilute aqueous solution at pairs of temperatures at which the solutions are isochoric do not show unique features. Taken together with previously published kinetic data, we conclude that special significance in the context of rates of chemical reaction in aqueous solutions should not be attached to the isochoric condition.

6.1 INTRODUCTION

6.1.1 THE ISOCHORIC CONDITION AND THE TEMPERATURE OF MAXIMUM DENSITY OF WATER

In 1935 Evans and Polanyi suggested that isochoric activation parameters for chemical reaction in aqueous solutions might be more mechanistically informative than conventional isobaric activation parameters; i.e. \[ \frac{\delta \ln(k)}{\delta T} \] \_\text{V} \text{ rather than } \[ \frac{\delta \ln(k)}{\delta T} \] \_\text{P} \text{ where } k \text{ is the rate constant for a spontaneous chemical reaction. They argued as follows: “Especial difficulty arises in solution, from the interaction between solvent and solute, which depends strongly on temperature. This effect would be to some extent eliminated by measuring the temperature coefficients at constant volume, instead of the current method of constant pressure.” They continued: “... which would eliminate the influence of thermal expansion of the solvent ...”}. Hence, isochoric enthalpies of activation can be envisaged to be more directly related to the temperature dependence of the reaction and therefore to the molecular details of the reaction under study without inclusion of the effect of temperature on the solvent. This proposal sparked enormous interest in several subject areas including ionic transport and chemical equilibria in solution.

With reference to chemical reactions in dilute aqueous solution, the isochoric (using “constant solvent molar volume” \( V_m \) as definition of the isochoric condition, vide infra) standard enthalpy of activation \( \Delta^\circ H_m^0 \) is related to the isobaric standard...
enthalpy of activation $\Delta^*H_p^0$ at temperature $T$ and the standard volume of activation $\Delta^*V_T^0$ via Equation 6.1 where $\alpha_{pl}^*$ and $\kappa_{T1}^*$ are the isobaric expansibility and isothermal compressibility, respectively, of water, $\Pi_{int}$ is the internal pressure of water.

$$\Delta^*H_{V_m}^0 = \Delta^*H_p^0 - T \cdot [\alpha_{pl}^* / \kappa_{T1}^*] \cdot \Delta^*V_T^0 = \Delta^*H_p^0 - (\Pi_{int} + p) \cdot \Delta^*V_T^0$$  \hspace{1cm} (6.1)

Baliga and Whalley\textsuperscript{5} noted that the dependence of $\Delta^*U_{V_p}^0$, a quantity related to $\Delta^*H_p^0$, on solvent mixture composition is less complicated than that of $\Delta^*H_p^0$ for solvolysis of benzyl chloride in ethanol + water mixtures at 298.125 K. A similar pattern was reported by Baliga and Whalley\textsuperscript{6} for the hydrolysis of 2-chloro-2-methylpropane in the same mixture at 273.15 K. Nevertheless, the significance of isochoric parameters in the context of both kinetic\textsuperscript{7} and equilibrium parameters\textsuperscript{8} has been extensively debated.\textsuperscript{9-11}

Much of the debate centred on the isochoric condition and the answer to the question 'what [is the] volume [that] is held constant?'.\textsuperscript{7,8} With reference to Equation 6.1, $\alpha_{pl}^*$ and $\kappa_{T1}^*$ depend on temperature. Then the volume identified by subscript $V_m$ on $\Delta^*H_{V_m}^0$ is dependent on temperature, rendering $\Delta^*H_{V_m}^0$ a local (in $p$ and $T$) quantity. Also, even if $\Delta^*H_p^0$ is independent of temperature, $\Delta^*H_{V_m}^0$ is clearly not. Further, the molar volumes of binary liquid mixtures (compositions in mole fractions) at fixed $T$ and $p$ (cf. kinetic data in reference 6) depend on composition so that within the context of $\Delta^*H_{V_m}^0$, the volume identified by subscript $V_m$ is not constant across a range of solvent mixtures.

In 1985, Haak \textit{et al.}\textsuperscript{7} reported kinetic data for the neutral hydrolysis of two 1-acyl-1,2,4–triazoles in binary aqueous mixtures. In their comments, Haak \textit{et al.}\textsuperscript{7} noted that at either side of the TMD of water there are pairs of temperatures where the molar volumes of water at ambient pressure are equal. Hence, rates of reaction for chemical reaction in dilute aqueous solution at such temperatures would yield pairs of isochoric rate constants. The suggestion was not taken up.

However, another controversy had arisen concerning chemical reactions in aqueous solutions at low temperatures. Hills and Viana\textsuperscript{12} reported that rate constants for hydrolysis of benzyl chloride in very dilute aqueous solutions increased with an increase in temperature above the TMD of water and, significantly, increased with a decrease in temperature below the TMD. Albery and
Curran\textsuperscript{13} were unable to confirm the pattern in rate constants reported by Hills and Viana.\textsuperscript{12}

Here, we report that rate constants for the hydrolysis of 1-benzoyl-1,2,4-triazole (6.1) in aqueous solution increase gradually as the temperature increases from below to above the TMD and that this is the normal pattern.

Having established the gradual increase in rate constant for hydrolysis of 6.1 around the TMD, the suggestion made by Haak \textit{et al.},\textsuperscript{7} concerning rates of reaction at pairs of temperatures for which the solvent molar volume is constant was taken up. For this study to be meaningful, the studied reaction had to satisfy at least three criteria;

(i) the rate of the chemical reaction can be precisely measured;  
(ii) the mechanism of reaction is well understood, and  
(iii) the rate of reaction is quite sensitive to temperature.

These three criteria are satisfied by the spontaneous first-order hydrolysis of 1-benzoyl-1,2,4-triazole (6.1) in aqueous solution. The mechanism of hydrolysis involves water-catalysed nucleophilic attack at the carbonyl group (Chapter 1 and the reaction scheme in reference 14).

The dependence of rate constants on temperature near the TMD of water is examined in terms of the dependence of the molar volume $V_m$ of water on temperature. Considering that a small amount of cyanomethane is introduced in the reaction medium, we did not use the data reported by Kell and Whalley\textsuperscript{15} and summarised by Kell\textsuperscript{16,17} but assumed a quadratic dependence of molar volume with temperature around the TMD as shifted by the presence of cyanomethane. It is shown that the actual choice of TMD is not important.

We show that the ‘isochoric condition’ does not reveal novel features concerning the hydrolysis reaction in aqueous solution. We draw attention to published kinetic data which support our conclusion but which, in the debate over isochoric kinetics, were overlooked.
6.2. RESULTS AND DISCUSSION

6.2.1 HYDROLYSIS OF 1-BENZOYL-1,2,4-TRIAZOLE AROUND THE TMD

First-order rate constants for the hydrolysis of 6.1 in aqueous solution were determined at 12 temperatures between 276.15 and 278.15K using a UV/Vis setup that had been specifically improved for monitoring reactions at stable low temperatures without condensation of water vapour (Table 6.1).

<table>
<thead>
<tr>
<th>Table 6.1: (Pseudo-) first-order rate constants for hydrolysis of 6.1a between 276.15 and 278.15K.a</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T / \text{K} )</td>
</tr>
<tr>
<td>276.15</td>
</tr>
<tr>
<td>276.25</td>
</tr>
<tr>
<td>276.45</td>
</tr>
<tr>
<td>276.65</td>
</tr>
<tr>
<td>276.95</td>
</tr>
<tr>
<td>277.05</td>
</tr>
<tr>
<td>277.25</td>
</tr>
<tr>
<td>277.35</td>
</tr>
<tr>
<td>277.45</td>
</tr>
<tr>
<td>277.65</td>
</tr>
<tr>
<td>277.85</td>
</tr>
<tr>
<td>278.15</td>
</tr>
</tbody>
</table>

(a) The first order rate constant at 298.15K is \( 21.2 \cdot 10^{-4} \text{ s}^{-1} \) from previous work (Chapter 5 and reference 14).

At three temperatures, \textit{viz.} 276.25, 276.45 and 277.25K, rate constants have been determined in triplicate. From the percentage-wise deviations from the individual average rate constants at each of these three temperatures, the standard deviation in the rate constants was determined to be 0.42%. From here on, the error in the rate constants has been taken to be 1.5 times the standard deviation.

We plotted both \( k \) and the molar volume of pure water, \( V_{m,1} \), as given by Kell,\textsuperscript{16} as a function of temperature (Figure 6.1). Please note that the molar volume and hence the concentration of water in pure water changes by less than 10ppm. Therefore, the temperature-induced change in concentration of water will not make a
detectable contribution to the change in observed (pseudo-) first-order rate constants.

![Figure 6.1: Plot of $k$ as a function of $T$ for the hydrolysis of 6.1 between 276.15 and 278.15K (o, left axis) and $V_{m,1}$ as a function of $T$ in the same temperature interval (-, right axis). Dotted line indicates the TMD.](image)

As evident from Figure 6.1, pairs of rate constants exist on both sides of the TMD for which the molar volume of water is constant. Hence, sets of rate constants have been determined under isochoric conditions.

It has to be noted that the actual TMD of the solutions in which the rate constants of hydrolysis have been determined is slightly lower than the TMD of pure water. This lowering of the TMD is caused by the introduction of cyanomethane in the reaction medium upon injecting the stock solution containing 6.1. In a typical experiment, 6±2 µl of a stock solution in cyanomethane is injected in 2.75 ml of water, resulting in a 0.126±0.042 mole% aqueous solution of cyanomethane. This leads to a lowering of the TMD\(^\text{18}\) by 0.28±0.09K to a TMD of 276.85±0.09K. In the following, we assume that the molar volume of the water/cyanomethane solution, $V_{m,1,2}$ varies quadratically with temperature around the TMD.\(^\text{19}\)

Plotting $\ln(k/T)$ against $T^{-1}$ produces a satisfactory linear Eyring plot (Figure 6.2) without an apparent break around the TMD.
The results are analysed using the Clark-Glew equation (Equation 6.2),

\[
\ln \left( \frac{k}{\theta} \right) = \ln \left( \frac{k(\theta)}{\theta} \right) + \left( \frac{\Delta^\neq H_p(\theta)}{R} \right) \left( \frac{1}{\theta} - \frac{1}{T} \right) + \left( \frac{\Delta^\neq C_p(\theta)}{T} \right) \left( \frac{\theta}{\theta} \right) - 1 + \ln \left( \frac{T}{\theta} \right) + \ldots \tag{6.2}
\]

The Clarke-Glew equation describes the isobaric dependence of rate constants on temperature around a chosen reference temperature \( \theta \) in terms of a series of thermodynamic parameters. The series can be extended by one thermodynamic parameter at a time until the statistical significance of the next derived thermodynamic parameter is unacceptable. Here, we have chosen the TMD of the aqueous solution of cyanomethane, \( \text{viz.} \ 276.85\text{K} \), as reference temperature. The results of the analysis are summarised in Table 6.2.

**Table 6.2:** Activation parameters of the hydrolysis of 6.1 in terms of the Clarke-Glew equation using 2 or 3 terms.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>excl. ( k(298.15\text{K}) )</th>
<th>incl. ( k(298.15\text{K}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 terms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( k(\theta) / 10^{-4}\text{ s}^{-1} )</td>
<td>4.106±0.006</td>
<td>4.106±0.006</td>
</tr>
<tr>
<td>( \Delta^\neq H_p(\theta) / \text{kJ mol}^{-1} )</td>
<td>49.8±1.6</td>
<td>50.4±0.6</td>
</tr>
<tr>
<td>3 terms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( k(\theta) / 10^{-4}\text{ s}^{-1} )</td>
<td>4.093±0.007</td>
<td>4.107±0.006</td>
</tr>
<tr>
<td>( \Delta^\neq H_p(\theta) / \text{kJ mol}^{-1} )</td>
<td>47.0±1.4</td>
<td>49.2±1.6</td>
</tr>
<tr>
<td>( \Delta^\neq C_p(\theta) / \text{J K}^{-1}\text{mol}^{-1} )</td>
<td>(1.66±0.47) ( 10^4 )</td>
<td>(1.1±1.8) ( 10^2 )</td>
</tr>
</tbody>
</table>
From Table 6.2, we conclude that $\Delta^\circ C_p(\theta)$ is not statistically significant for the present data set ($\Delta^\circ C_p(\theta)$ determined without $k(298.15K)$ is unrealistically large and has a considerable error margin, both as a result of the small temperature range whereas the value determined including $k(298.15K)$ is mainly dictated by $k(298.15K)$). Hence, we conclude that $k(\theta)$ equals $(4.106 \pm 0.006) \times 10^{-4}$ s$^{-1}$ and $\Delta^\circ H_p(\theta)$ equals $49.8 \pm 1.6$ kJ mol$^{-1}$, in close agreement with the literature value$^{14}$ at 298.15K of $47.2$ kJ mol$^{-1}$ (determined between 293.15 and 313.15K). This indicates that the isobaric standard enthalpy of activation is almost constant down to temperatures around the TMD of water. This is in contrast with the observation by Hills and Viana$^{12}$ that “… at one atmosphere the rate constant [for hydrolysis of benzyl chloride in water] as a function of temperature passes through a minimum at or near 40°C …”.$^{22}$

According to Figure 6.1, pairs of temperatures exist at which the molar volume of water is constant. Using a TMD of 276.85K and assuming the molar volume of the water/cyanomethane mixture to be a quadratic function of temperature around the TMD, we can plot the rate constant of hydrolysis as a function of $(T-TMD)^2$; Figure 6.3.

![Figure 6.3](image-url)

**Figure 6.3:** Plot of $k$ as a function of $(T-TMD)^2$, as proportional to the molar volume of the water/cyanomethane mixture, for the hydrolysis of 6.1 between 276.15 and 278.15K. Solid lines are calculated rate constants using Equation 6.2, $k(\theta)=4.106 \times 10^{-4}$ s$^{-1}$ and $\Delta^\circ H_p(\theta)=49.8$ kJ mol$^{-1}$.

According to Figure 6.3, activation parameters at constant molar volume of the solvent can be determined by taking the rate constants at different temperatures that correspond to the same solvent molar volume. The significance of the question ‘What volume is held constant?’ is emphasised by Figure 6.3, which shows that
despite the fact that isochoric sets of rate constants can be determined, the volume held constant is actually different for each set of isochoric rate constants. Essentially this is the case if isochoric activation parameters are determined at different temperatures and/or different solvent compositions. For three sets of data points, isochoric activation parameters can be determined directly. From the fit to Equation 6.2 and the assumption that \( V_{m,1,2} \) varies quadratically with temperature around the TMD, isochoric activation parameters around the TMD can be determined for different solvent molar volumes! New results do not follow from the use of Equation 6.2 combined with the notion that at pairs of temperatures on both sides of the TMD the molar volume of the water/cyanomethane mixture is identical. Considering that \( \ln(k/T) \) varies linearly with \( (T/K)^{-1} \) around the TMD, and that the isochoric sets of rate constants are on the isobaric plot, the actual temperature interval taken for the calculation of \( \Delta^o H_p \) is irrelevant and a value of 49.8 kJ mol\(^{-1}\) is consistently found. The equality of \( \Delta^o H_{p0} \) and \( \Delta^o H_p \) can be readily understood from the fact that the term \( \frac{T \cdot \alpha^*_{p1} / \kappa^*_{T1}}{p} \) in the temperature interval between 276.15 and 278.15K does not exceed 84 bar \((8.4 \times 10^6 \text{ N m}^{-2})\). Hence, assuming an isothermal volume of activation, \( \Delta^o V_{p0} \), of approximately \(-20 \times 10^{-6} \text{ m}^3 \text{ mol}^{-1}\) as determined for structurally similar activated amides at 298.15K,\(^7\) the second term on the left-hand-side of Equation 6.1 contributes less than \(+168 \text{ N m} \text{ mol}^{-1}\) or \(+0.168 \text{ kJ mol}^{-1}\). In fact, \( \left( \frac{d \ln V^*_{1}/dT}{p} \right) \) (cf. \( \alpha^*_{p1} \)) by definition equals 0 K\(^{-1}\) between isochoric points on both sides of the TMD, further clarifying the link between isobaric and isochoric enthalpies of activation around the TMD. In general, for \( T \) limiting to the TMD, it can be shown that the isobaric and isochoric enthalpy of activation are identical (Equation 6.3).

\[
\lim_{T \to \text{TMD}} \left( \Delta^o H_{p0} \right) = \lim_{T \to \text{TMD}} \left( \Delta^o H_{p} - T \cdot \left[ \alpha^*_{p1} / \kappa^*_{T1} \right] \cdot \Delta^o V_{p0} \right) = \lim_{T \to \text{TMD}} \left( \Delta^o H_{p0} \right)
\]

(6.3)

The above results are in line with kinetic results, obtained even before the Hills and Viana paper by Moelwyn-Hughes, Robertson and Sugamori.\(^{23}\) The latter determined the rate constants for hydrolysis of 2-methyl-2-chloropropane between 0 and 20\(^\circ\)C at 1\(^\circ\)C intervals in water without added cosolutes. Fitting their dataset to Equation 6.2, using \( \theta=\text{TMD}=277.13\text{K} \), yields the activation parameters as summarised in Table 6.3 (Figure 6.4).
Figure 6.4: Plots of $\ln(k/T)$ for the hydrolysis of 2-methyl-2-chloropropane between 274.15 and 293.16K. Left: $\ln(k/T)$ as a function of $(T/K)$, solid line indicates fit to Equation 6.2. Right: Residual as a function of $T/K$. Dotted lines indicate TMD. Data from reference 23.

Table 6.3: Activation parameters of the hydrolysis of benzyl chloride in terms of the Clarke-Glew equation using 3 terms.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k(\theta) / 10^{-3}$ s$^{-1}$</td>
<td>1.3735$\pm$0.0010</td>
</tr>
<tr>
<td>$\Delta^\theta H_p(\theta)$ / kJ mol$^{-1}$</td>
<td>101.6$\pm$0.2</td>
</tr>
<tr>
<td>$\Delta^\theta C_p(\theta)$ / J K$^{-1}$mol$^{-1}$</td>
<td>$(-3.3\pm0.3) \times 10^2$</td>
</tr>
</tbody>
</table>

The activation parameters are identical to those obtained by Moelwyn-Hughes, Robertson and Sugamori and, important for the present study, there is no indication of deviating behaviour at low temperatures (Figure 6.4). In addition, rate constants for hydrolysis reactions of numerous substrates have been determined down to temperatures below the TMD of water, in particular in the Robertson group. However, no atypical behaviour was observed, neither crossing the TMD of H$_2$O,$^{24-31}$ nor crossing the TMD of D$_2$O.$^{25-27,31,32}$ In combination with the present dataset and in light of the failure of Albery and Curran to reproduce the patterns observed by Hills and Viana,$^{13}$ there is no reason to believe that isobaric enthalpies of activation below the TMD are consistently different from those above the TMD.

6.2.2 THE SIGNIFICANCE OF ISOCHORIC ACTIVATION PARAMETERS

Now we return to the question of the significance of isochoric activation parameters. As cited before, Evans and Polanyi$^2$ expected that the effect of temperature on solvent-solute interactions could be “to some extent eliminated” by working under isochoric conditions as that “would eliminate the influence of thermal expansion of
the solvent”. From this suggestion, the isochoric condition as defined as “constant solvent molar volume” seems the most natural choice. However, care has to be taken in interpreting the isochoric activation parameters obtained using this definition for a number of reasons. First, under isochoric conditions, increasing temperature must be accompanied by an increase in pressure (for positive $\alpha_{p,1}^*$ and $\kappa_{T,1}^*$). Whereas the intermolecular distances in the solvent remain the same, “eliminating the influence of thermal expansion”, the internal energy of the solvent does not. With the changing internal energy of the solvent, the solvent changes. Especially in the case of water, with increasing temperature more and more directionally sensitive hydrogen bonds are broken despite the pressure increase keeping the molar volume of water the same. Hence, the interactions between solvent and solute will change as well. Whereas this effect might be small for solvents largely interacting through London dispersion interactions, which are normally more sensitive to distance than direction, for water it is expected to be significant. Second, as argued before, on the basis of the temperature dependence of $V_{1}^*$, $\alpha_{p,1}^*$ and $\kappa_{T,1}^*$, the isochoric condition is a local condition. Therefore, isochoric activation parameters as calculated at different temperatures are not isochoric with respect to each other. In other words, at different temperatures, the molar volume that is (mathematically) held constant is the molar volume of the solvent corresponding to that particular temperature and (in most cases) standard pressure. Third, the fact that isochoric activation parameters have been determined in solvent mixtures provides intriguing complexities. As argued above, $V_{m,1,2}^*$, $\alpha_{p,1,2}^*$ and $\kappa_{T,1,2}^*$ for solvent mixtures are dependent on composition. By the same line of argument as before for the temperature dependence of $V_{1}^*$, $\alpha_{p,1}^*$ and $\kappa_{T,1}^*$, isochoric activation parameters determined at individual solvent mixture compositions are not isochoric with respect to each other. In fact, if one is interested in intermolecular interactions in aqueous solution, it can be argued that it is the partial molar volume of water that should be held constant and not the total solvent molar volume (as is effectively done by using the isobaric expansibilities and isothermal compressibilities of the solvent mixture together with Equation 6.1).

Bearing in mind the issues mentioned above, a truly isochoric condition is readily available around the TMD of water. It has been shown that isochoric activation parameters can be determined using the fact that sets of temperatures exist at which the molar volume of water is equal. Datasets like these can even be
expanded by the application of pressure as the application of pressure results in a shifted plot of solvent molar as a function of temperature (Figure 6.5) leading to a complete range of temperatures corresponding to isochoric conditions.

![Figure 6.5](image)

**Figure 6.5**: Plots of $V_{m,1}$ for water between 273.15 and 283.15K at pressures of 1 bar (solid line), 2 bar (dotted line) and 4 bar (dot-dash line). Crosses indicate isochoric conditions. Data from references 15 and 16.

Figure 6.5 shows that even at moderate (i.e. experimentally non-problematic) pressures, a range of temperatures of at least 10K around the TMD satisfying isochoric conditions can be easily achieved. From Equation 6.3 and from the experimental results, however, it follows that for $T$ limiting to TMD, the isobaric and isochoric enthalpy of activation are equal. Therefore, using the natural isochoric conditions around the TMD to determine the isochoric enthalpy of activation does not yield additional insight.

### 6.3 Conclusions

Rate constants for hydrolysis of 6.1 have been determined between 276.15 and 278.15K. No atypical effects were found crossing the temperature of maximum density and neither were unusual effects found in a number of datasets available from literature. Isochoric activation parameters around the TMD of water have been determined at different solvent molar volumes. The isochoric activation parameters are equal to the corresponding isobaric activation parameters. We conclude that care has to be taken in interpreting isochoric activation parameters as they are only locally defined. These comments do not, of course, detract from the importance of the original proposal by Evans and Polanyi. We simply suggest that in many cases
where isochoric activation parameters have been reported, the proper meaning of the term ‘isochoric’ has not been recognised.

6.4 EXPERIMENTAL

6.4.1 KINETIC EXPERIMENTS

The hydrolysis reactions were followed using a Shimadzu Diode-array spectrophotometer, absorbances being recorded between 200 and 400 nm. The cell compartment of the spectrophotometer was thermostatted to a preset temperature using a Haake thermostatting unit equipped with a Pt100 electrode for direct control of the temperature of the cell block of the spectrophotometer. The temperature was checked and found to deviate by not more than 0.05 K from the preset temperature using a copper/constantin thermocouple during the kinetic experiments. Experience showed that control of temperature was improved by using ethanol rather than water as a bath liquid. We attribute the problems with using water to the formation of ice in the refrigeration unit.

A quartz cuvette, path length 1 cm, contained approx. 2.75 cm$^3$ of water of which the pH had been adjusted to 3.8 ±0.3 using HCl(aq). The cuvette was fitted with a stopper which had two small holes. The cuvette was thermostatted overnight. The cell compartment was sealed with cling film so that the air flow in the spectrophotometer did not effect the temperature of the cell compartment. The cell compartment was continually flushed with dry pre-cooled air.

The cell compartment was fitted with a hole in the cover through which wires and tubing could be led into the sealed cell compartment. Through this hole 1.5 cm$^3$ of solution in the sample cell was withdrawn. Between 4 and 8 µl of a stock solution containing 5 mg of 1-benzoyl-1,2,4-triazole in 1 cm$^3$ cyanomethane was injected into the cuvette. The solution in the cuvette was withdrawn and re-injected several times. Finally, the solution was injected into the cuvette very slowly in order to prevent the formation of air bubbles. After the reaction had been initiated, the temperature of the cell was monitored. Only after the temperature had returned to the required temperature were data points recorded of absorbance and time. During a given kinetic run the absorbances were measured during a brief exposure (1 second of exposure per minute) of the cell to the incident uv-visible beam of light. Poor kinetic data resulted if the solution was subjected to continuous radiation.
6.4.2 Materials

1-Benzoyl-1,2,4-triazole was synthesised according to published procedures.\textsuperscript{34} Water was distilled twice from an all-quartz distillation unit.
6.5 REFERENCES AND NOTES

(1) Part of this chapter is to be published: Blandamer, M. J., Buurma, N. J., Engberts, J. B. F. N. and Reis, J.C.R. in press.
(17) Note that volumes in references 15 and 16 are given both in cc (cm³) and ml, the latter being derived from the 1901 liter. The conversion between the 1901 liter and the present liter (equal to 1 dm³) is given by: 1 ml. = 1.000028 cm³.
(19) It turns out that the actual choice of the TMD for the present study is irrelevant. No differences emerge upon analysing the data around either a TMD set to 276.85K or set to 277.13K. In fact, both choices can be equally well defended.
(22) If $\Delta^\circ H(\theta)$ is determined independently below and above the TMD, the following numbers are found: For $\theta=$TMD=276.85: $k(\theta)=$(4.06$\pm$0.03) 10$^{-4}$ s$^{-1}$ (below TMD), (4.08$\pm$0.02) 10$^{-4}$ s$^{-1}$ (above TMD) and (4.10$\pm$0.01) 10$^{-4}$ s$^{-1}$ (above TMD, incl. 298.15K); $\Delta^\circ H(\theta)=32.2$±8.2 kJ mol$^{-1}$ (below TMD), 57.4±2.6 kJ mol$^{-1}$ (above TMD) and 50.7±0.6 kJ mol$^{-1}$ (above TMD, incl. 298.15K). For $\theta=$TMD=277.13: $k(\theta)=$(4.17$\pm$0.02) 10$^{-4}$ s$^{-1}$.
(below TMD), (4.18±0.02) 10^{-4} \text{s}^{-1} (above TMD) and (4.20±0.01) 10^{-4} \text{s}^{-1} (above TMD, incl. 298.15K); Δ^\neq H(\theta)=42.6±3.2 \text{kJ mol}^{-1} (below TMD), 58.8±4.0 \text{kJ mol}^{-1} (above TMD) and 50.6±0.6 \text{kJ mol}^{-1} (above TMD, incl. 298.15K). Considering the strongly different results upon changing the exact value of the TMD (276.85 or 277.13K) or depending on whether or not the rate constant at 298.15K is included, we contend that the apparent difference in Δ^\neq H (\theta) is caused by the individual data sets being too small. Admittedly, this can be ameliorated by extending the dataset.

(33) It has to be noted that despite the fact that Figure 6.5 contains data points on a line indicating the isochoric condition, isochoric activation parameters as calculated for the individual datapoints are different as the Tαp/κT terms varies around the TMD. This once more stresses the local character of calculated isochoric activation parameters.